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S/N 08/991,143

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Bianca M. Conti-Fine	Examiner: Patrick J. Nolan
Serial No.:	08/991,143	Group Art Unit: 1644
Filed:	December 16, 1997	Docket: 600.423US1
Title:	METHODS TO TREAT UNDESIRABLE IMMUNE RESPONSES	

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Bianca Conti-Fine, M.D., declare and say as follows:

1. I am the inventor of subject matter claimed in the above-identified application. In the Office Action dated December 4, 2000, the Examiner rejected claims 1-13, 16-18, 31, and 34-39 under 35 U.S.C. § 112, first paragraph, as the specification allegedly fails to enable a method of using universal epitope peptides other than a method to treat experimental autoimmune myasthenia gravis in mice with acetylcholine receptor peptides.

2. However, as the specification in the above-identified application makes clear, antigens other than the acetylcholine receptor have universal epitopes and so may be employed in the methods of the invention. One such example is the use of universal epitope peptides from factor VIII to treat hemophilia. Approximately 25% of patients with severe hemophilia A develop blocking antibodies (inhibitors) to the missing coagulation factor, factor VIII (fVIII). These inhibitors block fVIII activity and significantly compromise the ability to achieve therapeutic homeostasis during bleeding episodes. FVIII inhibitors also develop also during autoimmune hemophilia A, a rare but frequently fatal disease in which fVIII is the target of an autoimmune response. Hemophilia A results from a genetic defect in the fVIII gene while acquired (autoimmune) hemophilia is the result of an autoimmune response to fVIII. FVIII inhibitors are high affinity IgG, and their synthesis requires CD4+ T helper cells specific for fVIII.

3. An art-recognized mouse model of hemophilia A is available to study the efficacy of fVIII peptides. These mutant mice (hereinafter "hemophilia A mice"), which have a targeted gene disruption of the fVIII gene that results in severe fVIII deficiency, are an excellent model of

hemophilia A, as these mice develop fVIII inhibitor antibodies and a $CD4^+$ response after intravenous (i.v.) exposure to human fVIII. The concentration of serum anti-fVIII antibody in these mice increases with the number of exposures to fVIII and the dose used. Approximately 50% of the hemophilia A mice treated with human fVIII i.v. have a detectable proliferative response of spleen T cells.

4. A group of 8 hemophilia A mice was treated with a peptide pool having immunodominant epitopes from the A1 domain, A2 domain, C1 domain and C2 domain of human fVIII. The mice received 50 μg of each peptide in the pool twice a week for three weeks before the beginning of the intravenous administrations of human fVIII. A second group of 7 control mice was treated nasally with PBS only. After the beginning of the treatment with fVIII, the peptides (or PBS only) were administered nasally only once per week. Each mouse received 1 μg of fVIII intravenously every two weeks for a total of up to nine injections. The mice that were treated nasally with the fVIII peptides received intravenous injections of fVIII mixed with the epitope peptide pool (25 μg of each peptide in each injection). The control mice (sham treated with PBS) received intravenous administrations of fVIII without any peptide. Blood was obtained from the mice two weeks after each intravenous injection of fVIII.

5. Mice treated with fVIII and sham tolerized with PBS produced anti-fVIII IgG antibodies. Of the mice treated with fVIII and tolerized with fVIII peptides, one mouse developed modest anti-fVIII antibodies, while the remaining mice developed transient, minimal amounts of anti-fVIII antibodies. Moreover, none of the mice exhibited any adverse symptoms associated with peptide administration. Thus, fVIII peptide therapy reduced levels of anti-fVIII antibodies in fVIII treated hemophilia A mice.

6. Healthy humans have recurrent, transient sensitization of CD4+ cells to fVIII. This is likely due to extravasation of fVIII at sites, such as bruises, where fVIII sequence may be presented by professional antigen presenting cells, able to prime potentially autoreactive CD4+ cells specific for fVIII epitopes. In normal individuals, who have high blood levels of fVIII, the activated anti-fVIII CD4+ cells quickly disappear, possibly as a result of anergy or deletion by

peripheral mechanisms of tolerance. Such cells persist in hemophilia A patients because their low fVIII levels, even after therapy, do not suffice for tolerization. Thus, the presence of anti-fVIII CD4+ cells in healthy humans, as well as those with hemophilia A, is useful to identify universal CD4+ epitopes for fVIII in humans.

7. To identify the CD4+ epitope repertoire on fVIII in humans, CD4+ cells from hemophilia A patients, autoimmune hemophilia patients, and healthy individuals that have a CD4+ response to fVIII were employed in at least one of three assays: 1) identification of the epitope repertoire of unselected CD4+ cells from the patient's blood by proliferation experiments using CD8+ depleted, CD4+ enriched peripheral blood lymphocytes (PBL) challenged with each individual peptide; 2) identification of the CD4+ subset (Thl, Th2 or Th3) recognizing the different fVIII epitopes, by immunospot assays of the cytokines secreted by individual blood CD4+ cells in response to challenge with the difference fVIII peptides (preferably, IL-2 and γ-interferon are employed to detect Thl cells, and IL-4 and IL-5 are employed to detect Th2 cells); and 3) propagation of fVIII-specific CD4+ lines, by cycles of stimulation *in vitro* of the PBL with fVIII followed by IL-2 or IL-4, and determination of their epitope repertoire and the Thl, Th2 or Th3 subset involved in the anti-epitope response, by challenging them with individual synthetic sequences in proliferation and immunospot assays.

8. Using such assays, sequence segments of the A3 and C2 domains of fVIII that were recognized by all hemophilia A patients with inhibitors were identified. Also, based on the structural similarity between A2 and A3 domains and the relative location of universal epitopes with the sequence regions forming binding sites for inhibitors, regions of the A2 domain that are likely to form universal CD4+ epitopes were identified. A pool of those universal epitope sequences is useful to induce tolerance to fVIII in hemophilia A patients and in patients with acquired hemophilia.

9. These results provide further evidence that universal immunodominant epitopes are generally present on antigens and that peptides having those epitopes are useful to inhibit an indication or disease associated with aberrant, pathogenic or undesirable antibody production,

e.g., to inhibit the amount of anti-fVIII antibodies associated with fVIII therapy for hemophilia A.

10. With respect to the speculation in Wraith et al. (Cell, 59, 247 (1989)) that the inhibition of one class II molecule may lead to escape to an autoimmune response to a separate epitope restricted by a different class II molecule, the administration of a universal epitope peptide for antibody-mediated disorders does not necessarily lead to deletion of peptide-specific T cells but does lead to stimulation of modulatory T cells. These modulatory T cells (e.g., Th2 and Th3) exert their suppressive action on an antibody-mediated immune response by virtue of the regulatory cytokines that they secrete. Those cytokines inhibit the activity of any pathogenic immune cells in their proximity, irrespective of their epitope specificity, or even antigen specificity (antigen-mediated bystander suppression). This protective mechanism is quite different from those of epitope-specific immunosuppressive approaches that act through direct deletion of pathogenic T cells. Those approaches require the use of a most comprehensive pool of epitopes and potential epitopes, and they stand the chance of being ultimately ineffective because of the emergence of new epitope specificities.

Antigen-specific immunosuppressive methods that stimulate modulatory T cells do not incur those risks, and do not require the use of an extensive epitope pool. This is because sensitization of modulatory T cells to one or more, e.g., a few, dominant epitopes on an antigen inhibits the activity of any nearby pathogenic T cells, even if those nearby cells recognize other epitopes or other antigens. The epitope specificity of the modulatory T cells works to target them into the organ or tissue where their action is beneficial.

11. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 6/1/2001

By: Bianca Conti-Fine